

Rodlet cells in the alimentary tract of three genera of lizardfishes (Synodontidae, Aulopiformes): more on these enigmatic “gate-guards” of fishes

by

Lev FISHELSON* (1), Barry RUSSELL (2), Daniel GOLANI (3) & Menachem GOREN (1)

ABSTRACT. - Rodlet cells (RCs) of the alimentary tract were studied in lizardfishes (Synodontidae) of the three major genera: *Synodus*, *Trachinocephalus* and *Saurida*, collected from the Mediterranean, the Red Sea, the South-African coast and the Great Barrier Reef (GBR), Australia. In the studied species two forms of RCs were revealed: one elongated, resembling those previously described from other fishes; and a second, bulbous in form, which was discovered in two species only, *Saurida filamentosa* and *S. argentea* from the GBR. In all species studied the cells are situated in the mucosal epithelium: the elongated form close to the luminal surface of the epithelium, and the bulbous form deep among the other cells. The two forms also differ in their microstructure, and their different positions in the epithelium activate different paths for release of the rodlets into the lumen. Each cell contains 15-30 rodlets, which change from round to needle-shaped during maturation, and are later released into the gut lumen. The development of the rodlets and the possible role of the RCs are discussed.

RÉSUMÉ. - Les cellules à bâtonnets dans les voies alimentaires de trois genres de poissons lézard (Aulopiformes, Synodontidae) ; des éléments supplémentaires sur ces énigmatiques “gardiens de portes” des poissons.

Les cellules à bâtonnets (RCs) des voies alimentaires ont été étudiées dans les trois genres majeurs de Synodontidae : *Synodus*, *Trachinocephalus* et *Saurida*, recueillis en mer Méditerranée, en mer Rouge, sur la côte d’Afrique du Sud et de la Grande Barrière de Corail (GBR) en Australie. Dans les espèces étudiées, deux formes de RCs sont observées : une allongée, qui ressemble à celles qui ont été décrites antérieurement chez d’autres espèces et une seconde forme bulbeuse, trouvée seulement chez deux espèces, *Saurida filamentosa* et *S. argentea* de GBR. Chez toutes ces espèces, les cellules sont situées dans l’épithélium muqueux : la forme allongée proche de la surface luminale de la muqueuse et la bulbeuse en profondeur parmi les cellules muqueuses. Les deux formes diffèrent aussi dans leurs microstructures et leurs différentes positions dans l’épithélium activent des chemins différents concernant la libération des bâtonnets à l’intérieur du lumen. Chaque cellule contient 15 à 30 bâtonnets qui, pendant la maturation, se transforment d’une forme ronde à une forme plus aculéiforme et ensuite sont libérés dans la lumière intestinale. Le développement des bâtonnets et le rôle possible des cellules à bâtonnets sont discutés.

Key words. - Synodontidae - Lizardfish - Alimentary tract - Rodlet cells - Structure - Development.

The study of rodlet cells (RCs) found in the epithelial layers of various fish organs offers an exceptional example of a scientific controversy, in which different researchers offer different explanations for the same phenomenon. To begin with, some historical facts: the RCs were first described by Thélohan (1892) and Laguesse (1895) shortly afterwards named them *Rhabdospora thelohani*, placing them as a coccidian parasite of the Apicomplexa order. This cell is prominent in its strong cover and small, dense bodies (rodlets) of a proteinous nature, situated in the cell cytoplasm (Fig. 1). Each rodlet is surrounded by a sac, which according to various authors is of a glycoproteinous nature (e.g., Bielek, 2002). Following the initial description and until very recently, some researchers continued to recognize the RCs as unique “inhabitants” or parasites of the

teleost fish skin and internal tissues (e.g., Mayberry *et al.*, 1979; Agulleiro *et al.*, 1986), while others perceived them as specialized granular eosinophilic or inflammatory cells, forming an endogenous part in the immune-system that “safe-guards” the fish from parasitic organisms or harmful chemical substances (Manera and Dezfuli, 2004; Silphaduang *et al.*, 2006; Dezfuli *et al.*, 2007a, 2007b). Reite (2005) coined the term “standing force” for them, in combating the parasitic helminthes. Densmore *et al.* (2007) in contrast, did not find a correlation between parasites in fish and the occurrence of RCs. Barber *et al.* (1979) showed that these rodlets do not possess RNA and that their DNA seems to differ from that of their rodlet-cell nuclei. Viehberger and Bielek (1982), using various methods of labeling, argued that the rodlets consist in condensed DNA “with extraordinary con-

(1) Dept. of Zoology, Tel Aviv University, Tel Aviv 69978, Israel. [gorenm@post.tau.ac.il]

(2) School of Environmental and Life Sciences, Charles Darwin University, Darwin, Australia. [barry.russell@nt.gov.au]

(3) Dept. of Evolution, Ecology & Behavior, Hebrew University, Jerusalem, Israel. [dgolani@cc.huji.ac.il]

* Corresponding author [fishelv@post.tau.ac.il]

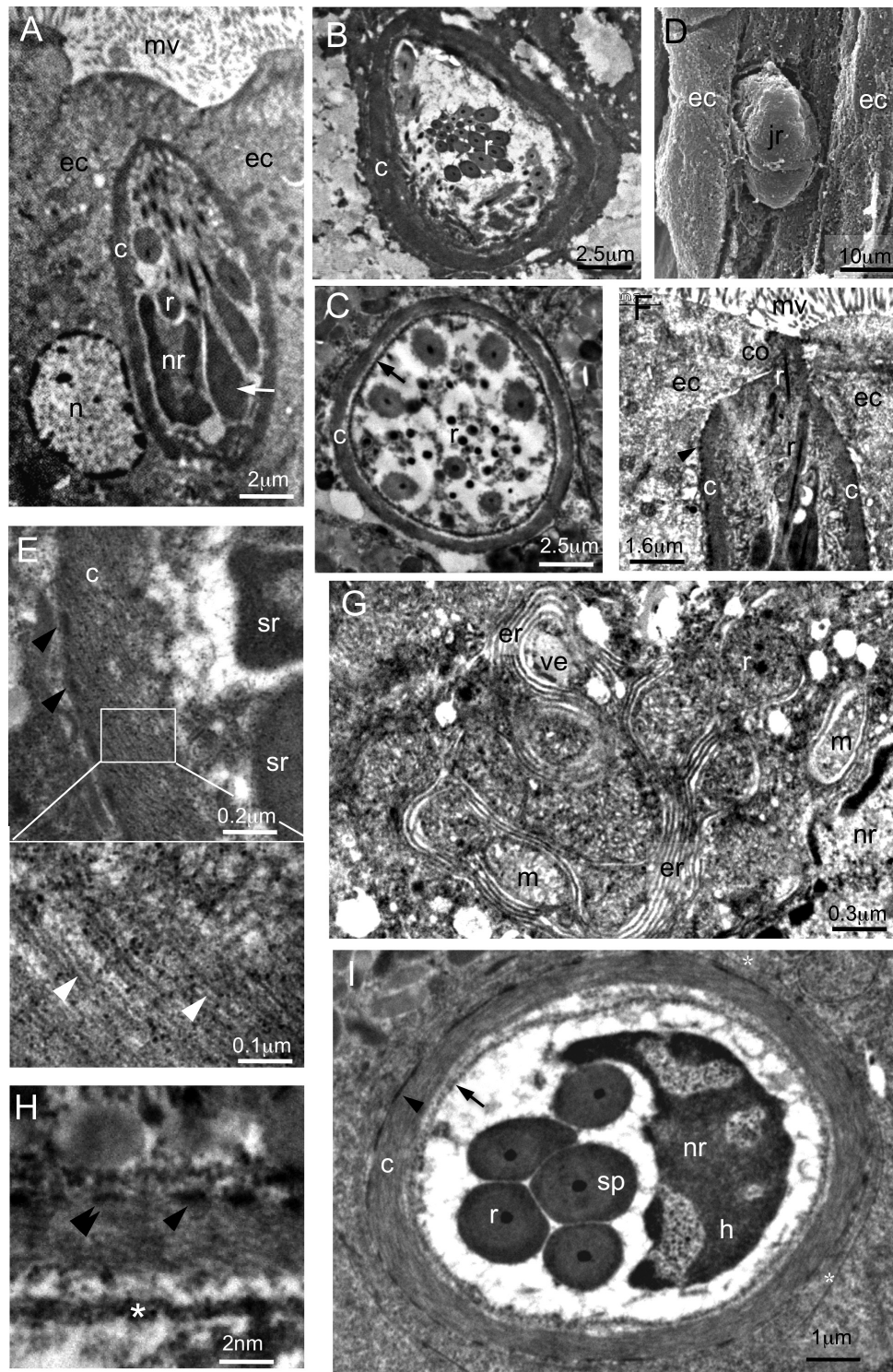


Figure 1. - SEM and TEM of rodlet cells. **A:** Longitudinal section of a rodlet cell of *Saurida tumbil*; **B:** *Ibid.*, of *Saurida filamentosa*; **C:** Cross-section of rodlet cell of *Saurida argentea*; **D:** immature rodlet cell of *S. filamentosa*; **E:** Section of rodlet cell capsule of *Synodus variegatus* (inset, enlarged rectangle of the same); **F:** Apical part of rodlet cell of *Sauria macrolepis*; **G:** ER and mitochondria in rodlet cell of *Saurida tumbil*; **H:** Section of a rodlet cell capsule of *Synodus variegatus*; **I:** Cross-section of rodlet cell of *S. macrolepis*. c, rodlet cell capsule; co, conoid; ec, epithelial cells; er, endoplasmic reticulum; h, nuclear heterochromatin; jr, immature rodlet cell; mv, microvilli; n, nucleus of epithelial cell, nr, nuclei of rodlet cell; r, rodlet; sp, two layers in a rodlet; sr, sac of rodlet; ve, vesicles of ER; black arrow and aster, inner layer along the capsule of rodlet cell; white arrow, sac of rodlet; double arrow, fragmented ER tubules; black arrowhead, opaque cut section of filaments; white arrowhead, microtubules.

figuration", while Barber and Mills Westermann (1986a), following the bleaching of rodlets by EDTA and DNase digestion, strongly suggested their DNA content. Fishelson and Becker (1999) revealed that not all specimens of a single species possess these cells. The studies by Schmachtenberg (2007) and Bielek (2008) are also less conclusive; indeed, the majority of pertinent studies on this issue have questions that have remained at least partly open: namely, are the RCs "unique parasites" specific to bony fish, or are they a unique evolutionary invention of the non-specific fish immune system? The first review on RCs was published by Mayberry *et al.* (1979) and the most recent ones by Manera and Dezfuli (2004) and Bielek (2008). These enigmatic cells have been observed to date in different organs of various species of bony fish belonging to ca. 35 families, found in fresh as well as marine waters (Manera and Dezfuli, 2004). The latter review summarizes most of the current knowledge on the distribution of RCs in the various organs of fishes, including an extensive list of the pertinent literature. The more recent studies on these cells have revealed their existence also in the sensory systems of the zebrafish (Dezfuli *et al.*, 2007b).

The present study deals with species from the three major genera, *Saurida*, *Synodus* and *Trachinocephalus*, of lizardfishes (Synodontidae) collected from various regions (Tab. I). It forms a natural continuation of our study of the alimentary tract of these fishes (Fishelson *et al.*, 2011), and our earlier interest in the RCs (Fishelson and Becker, 1999). In this latter study we suggested that the endothelial cells of the fish are invaded by an "external organism" that uses them as host and carrier, an idea also adopted by Schmachtenberg (2007).

The lizardfishes (Synodontidae) are lie-in-wait predators of up to 600 mm standard body length (SL), feeding mainly on fishes. They are found from the most shallow, subtidal waters, to around 800 m depth, and in some regions are commercially important (Golani and Ben-Tuvia, 1995).

Table I. - Data on the studied lizardfishes. AU: Australia; GBR, off Great Barrier Reef; SRS: southern Red Sea; GA: Gulf of Aqaba; RS: Red Sea; EM: East Mediterranean; MS: Mediterranean Sea; SL: standard length (in mm); *: also recognized as *Saurida undosquamis*.

Species	SL	No. of specimens	Origin
<i>Saurida macrolepis</i> (Tanaka, 1917)*	45-320	10	RS, EM
<i>Saurida argentea</i> (Macleay, 1881)	182-230	2	AU (GBR)
<i>Saurida filamentosa</i> Ogilby, 1910	180-210	3	AU (GBR)
<i>Saurida undosquamis</i> (Richardson, 1848)	325, 360	2	AU (GBR)
<i>Saurida tumbil</i> (Bloch, 1975)	35-470	5	SRS, GA
<i>Saurida gracilis</i> (Quoy & Gaimard, 1924)	128-109	3	Taiwan
<i>Synodus variegatus</i> (Lacepède, 1803)	60-145	6	GA, RS
<i>Synodus kaianus</i> (Günther, 1880)	110, 120	2	Hawaii, Malokai
<i>Synodus saurus</i> (L., 1758)	110-165	4	MS
<i>Trachinocephalus myops</i> (Forster, 1801)	86-115	6	GA

MATERIALS AND METHODS

The studied lizardfishes were collected along the Israeli part of the Eastern Mediterranean, the Red Sea, and off the Great Barrier Reef of Australia. Well preserved specimens were also donated by various museums (Tab. I). Freshly collected fishes were preserved on ice until processing. The alimentary tract was dissected out and fixed in 3.5% glutaraldehyde+1% formaldehyde for electron microscopy (EM), and in Bouin solution for light microscopy (LM). Following 12 h fixation, samples for LM were washed in water and ascending grades of ethanol, followed by dehydration and embedding in paraplast. The 8-10 μ m sections from these blocks were stained with hematoxylin-eosin, Masson's trichrome, toluidine, and cresyl-violet. The sections were studied with a Lieder microscope; photos were taken with a Dino digital camera attached to the PC. For electron microscopy (EM), the samples were divided into two parts: one for transmission electron microscopy (TEM) and the second for scanning electron microscopy (SEM). The samples for TEM were post-fixed in osmium-tetroxide and embedded in Epon. Blocks of this material were cut and the resulting serial microsections were stained with uranyl acetate and lead citrate, studied with a JEOL 1200 EX for TM-micrographs and photographed with a built-in digital camera. For SEM, the samples passed ascending grades of ethanol, and were then CO₂ saturated, point-dried, gold-plated and studied with a JEOL 840 A, with a built-in ADDA (Olympus) digital camera for microphotography. The number of RCs/section of gut was calculated by counting them from 6-8 cross-sections.

RESULTS

Form of the rodlet cells

Rodlet cells (RCs) were found along the alimentary tract of all the studied species of lizardfishes, especially dense in the stomach wall, situated within the mucosal epithelium (Fig. 1A) and, except for a small junction at their apical part, without clear desmosomal junctions to the surrounding epithelial cells along the epithelium. Two forms of rodlet cells were observed in the studied lizardfishes: one elongated (Fig. 1A), in which the long axis of the cell is 2.5-3.5-fold longer than the short one. Similar RCs were observed in numerous fish species, and revealed by us in specimens of all three genera of

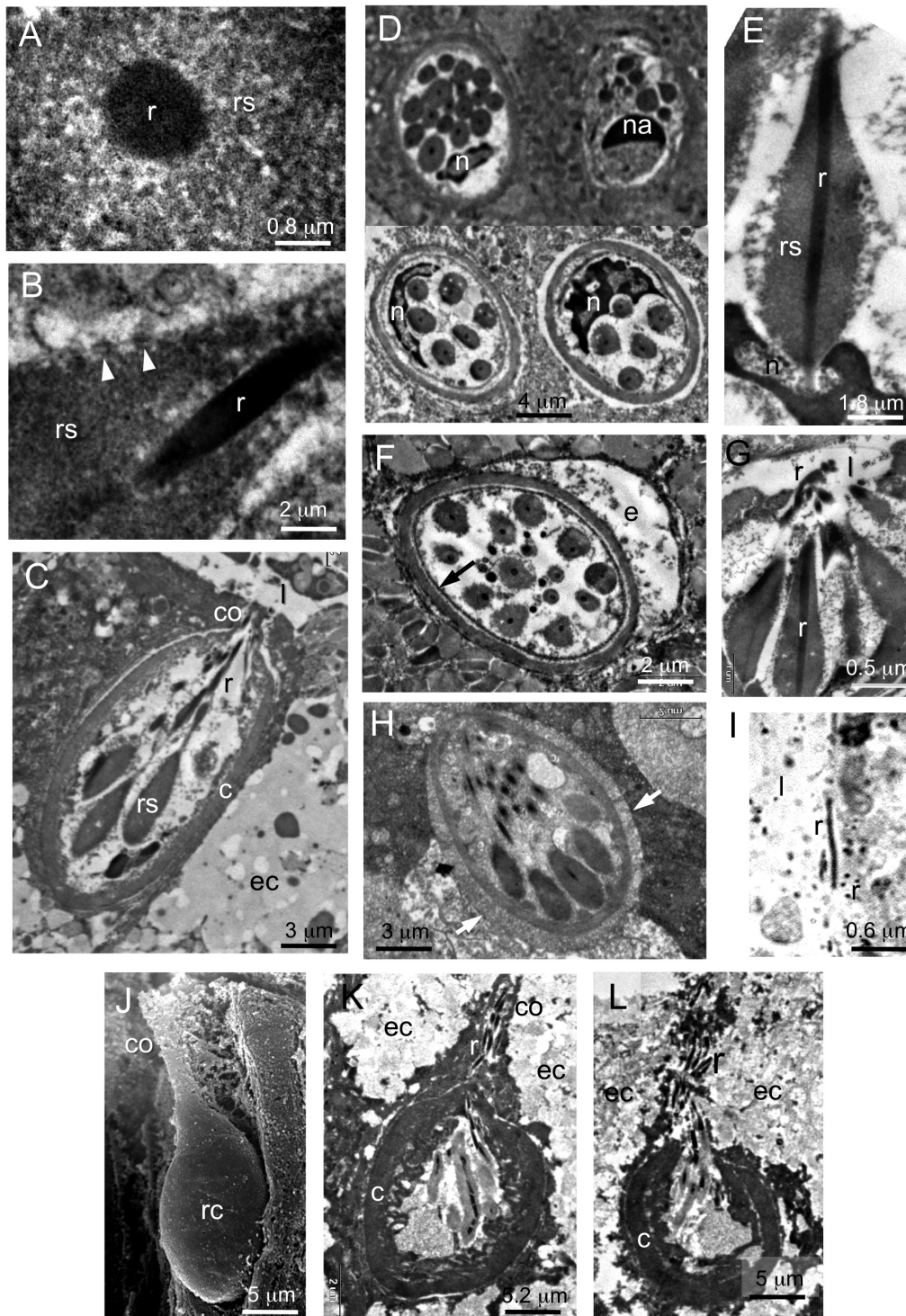


Figure 2. - Maturing RCs. **A:** Cross-section of a rodlet and its sac of *Saurida undosquamis*; **B:** Longitudinal section of a rodlet and its membrane of *S. undosquamis*; **C:** Longitudinal section of rodlet cell of *Saurida macrolepis*; **D:** Cross sections of four RCs (upper right, shrinking (apoptotic nucleus) of *S. macrolepis*; **E:** Longitudinal section of rodlet “sitting” on the rudiments of nucleus of *Synodus saurus*; **F:** Section of a displaced rodlet cell of *Saurida filamentosa*; **G:** Apical part of a rodlet cell of *Saurida tumbil*; **H:** Rodlet cell of *S. macrolepis*; **I:** Rodlets and their fragments in the stomach lumen of *Synodus kaianus*; **J:** Apical neck formation in rodlet cell of *S. filamentosa*; **K, L:** Needle-shaped rodlets along the capsule extension and liberated of *S. filamentosa*. c, capsule; co, “conoid”; e, empty space; ec, epithelial cells; l, lumen of stomach; m, mitochondria; n, nucleus; na, nucleus in apoptosis; r, rodlets; rc, rodlet cell; rs, sac of a rodlet; black arrow, inner layer of rodlet cell; white arrow, an outer envelope of the rodlet cell capsule; white arrowhead, membrane of rodlet sac apposed tightly to ER membrane.

lizardfishes (*Saurida*, *Synodus*, *Trachinocephalus*) from the Mediterranean Sea, the Red Sea and the South-African coast. These RCs are situated between the apical parts of cells of the mucosal epithelium, with their slightly pointed collar-like apex almost exposed to the alimentary tract lumen. The second, different, form of RCs was revealed in *Saurida filamentosa* and *S. argentea* collected off the Great Barrier Reef. Their RCs are of a bulbous form and upon maturation they develop an elongated neck (Fig. 1B, C). In most instances such RCs were found situated deeply among the epithelial cells, closer to the basal membrane of the epithelium. The RCs described from *Phoxinus* by Dezfuli *et al.* (2000) bear slight resemblance to these cells.

In the studied lizardfishes the immature RCs were of 3–4 μm , and on some sites some of these were found in the stomach, bordering the gastric gland-layer (Fig. 1D).

In most species of fish studied, with growth such immature RCs change in form: from oval, rounded at the apical ends, and rounded or round in cross-section, to elongate when mature, tapering toward the intestinal lumen (Fig. 1A). Such mature and elongated cells are circa 12–14 μm in length and 4 μm in diameter. An exception was found in *Saurida tumbil* and in some *S. macrolepis*, in which mature RCs attained up to 20 μm in length and 6–8 μm in diameter. However, in *S. filamentosa* and *S. argentea* the round immature RCs retained bulbous throughout growth attaining only 10–12 μm in length and diameter (Fig. 1B, C). Finally, such RCs develop a long apical “neck” (Fig. 2J)

The cytological structure of the RCs in the lizardfishes resembles that of these cells in other fish families, with two prominent features: the cell is surrounded by a thick membrane or capsule, attached inside with the host-cell *plasmalemma* and the cytoplasm of the cell bears a number of opaque bundles of rodlets, each embedded in a less opaque sac (Fig. 1A, C).

The rodlet cell capsule

In immature RCs of 3–4 μm in diameter the encapsulating membrane is 8–10 nm thick, closely merging with the cell *plasmalemma*; in mature RCs the cell membrane is buttressed by a fibrillar capsule, ca. 400 nm thick (Fig. 1B, E) in the elongated RCs, and up to 500 nm thick in the bulbous RCs. This capsule, in addition to filaments, features longitudinal bundles of microtubules (Fig. 1E and inset, rectangle of E). Each of these tubules is 12–14 Å, extending partly parallel to the long axis of the cell, and partly winding, ending at the cell membrane. Cross-sections of such end-points of the tubules reveal opaque plugs (Fig. 1E, H, I).

The inside of rodlet cells

The cytoplasm of the RCs is rich in endoplasmic reticulum (ER) (Fig. 1G), a few mitochondria and a large, basally-situated ellipsoid nucleus, prominent in the opaque hetero-

chromatin along its membrane (Fig. 1A). The first miniature rodlets appear in RCs of 3–4 μm (Fig. 1G), surrounded by a closely-attached membrane (Fig. 1H), which according to Bielek (2008), is part of the ER network. With cell growth, the rodlets increase in dimensions, attaining up to 2 μm in cross-section, closely attached to the cell nucleus (Fig. 1I). Each rodlet is composed of a dense mass of dark granular bodies (Fig. 2A), frequently forming tubular structures. Toward their periphery the density of the dark rodlets decreases, with their dispersion into the less opaque cytoplasm of the rodlet-sac, often revealing two layers of different densities (Fig. 1I). Each rodlet sac is surrounded by a membrane, often aligned with membranes of the endoplasmic reticulum (Fig. 2B). The number of rodlets increases with growth of the cell, and the rodlets gradually change from round to elongate (Fig. 1F, 2C, E). In parallel, their sacs inflate, elongating to match the rodlet form, and becoming less opaque. Concomitantly, the RCs nuclei change from oval to irregular, and their rim of opaque heterochromatin narrows, leaving small opaque sites only (Fig. 2D). Some of these nuclei seems to demonstrate various stages of apoptosis (Fig. 2D, upper left). In numerous instances, the form and dimensions of the rodlets were observed to match the irregularities observed in the adjacent nucleus as if indenting it (Fig. 1I, 2D). Rodlets were also frequently observed to be “attached” to the remnants of the nucleus (Fig. 2E, G). In ripening RCs the number of such rodlets increases from 3–4 to 23–30 in the Australian *Saurida filamentosa*, and parallel to this the cell nucleus undergoes “shrinkage” in size and finally disappears (Fig. 2C, F).

With maturation of the rodlets and their strong elongation, their sacs too strongly elongate. The apical ends of these rodlets conjoin and progress toward the stomach and intestinal lumen (Fig. 2C, G). Parallel to this the RCs cytoplasm becomes more translucent, and the ER forms vacuoles and disintegrates. At this stage an internal opaque rim, of unclear origin, forms along the inside of the cell capsule (Fig. 1C, 2F) and outside it (Fig. 2H). The rodlets, when mature for release, are all needle-shaped, 1–1.3 μm long (Fig. 2C, E), frequently observed at the opening into the lumen or outside it, often fragmenting into small particles at the opening (Fig. 2G, I). In *Synodus variegatus*, *Trachinocephalus myops* and *Saurida macrolepis* the apical part of the RCs is situated close to the lumen and forms a short cytoplasmic “neck” that resembles the conoids of coccidian parasites (Fig. 2C).

However, in maturing bulbous RCs of *Saurida filamentosa* and *S. argentea*, situated deep among the epithelial cells, an extension of the “conoid” gradually develops that penetrates apical among the epithelial cells (Fig. 2J, K). Inside and along such extensions the needle-shaped rodlets protrude toward the alimentary tract lumen (Fig. 2L, M). At the apical ends of such mature RCs their capsule wall degrades, becoming thinner and irregular (Fig. 3A), and finally opens

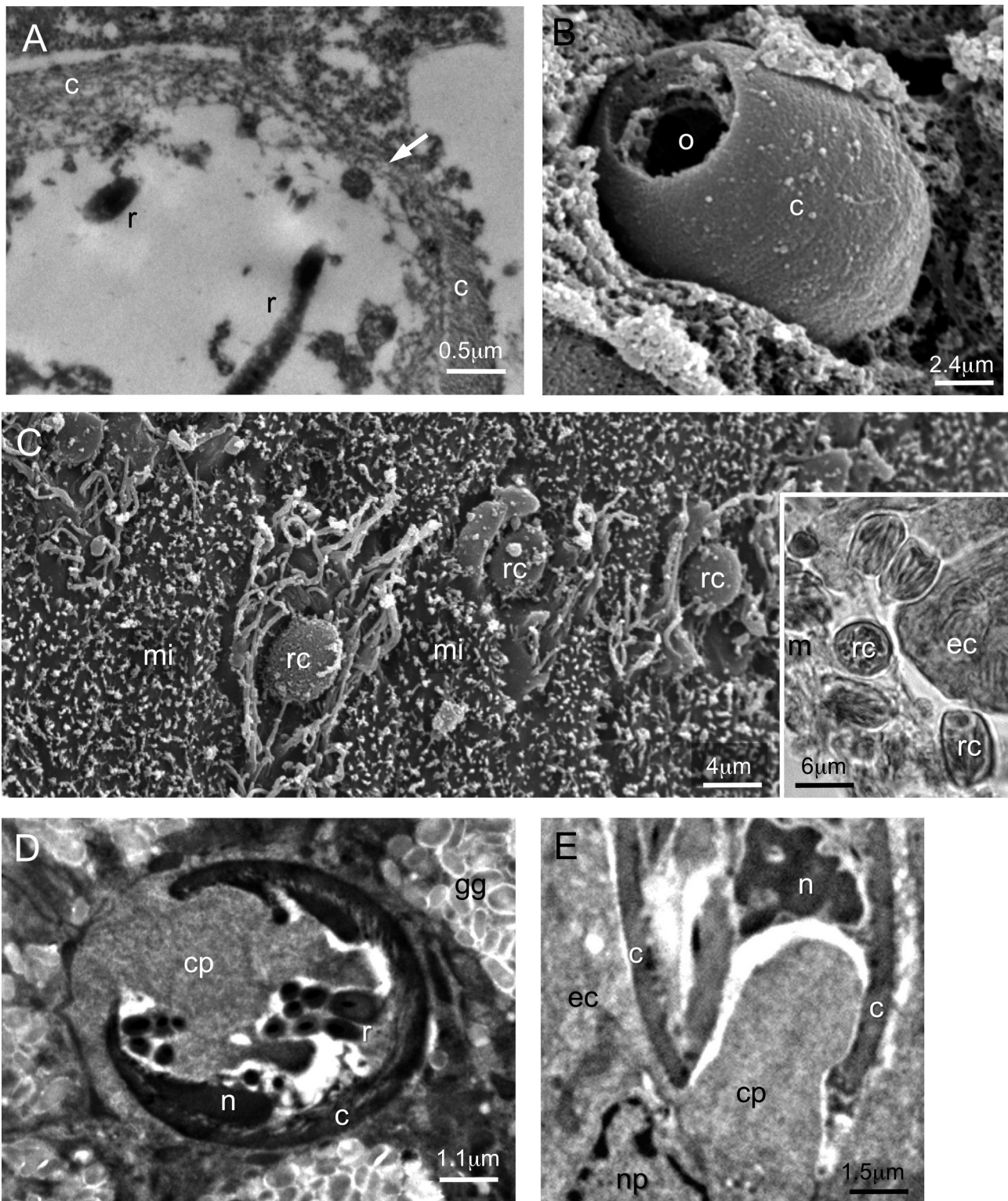


Figure 3. - Parts of RCs. **A:** Weakened apical membrane in rodlet cell capsule of *Saurida macrolepis*; **B:** Empty rodlet cell capsule of *Saurida filamentosa*; **C:** RCs exposed on stomach inner surface of *Saurida tumbil* (inset, free RCs in the gut); **D:** Penetration of phagocyte cytoplasm into rodlet cell of *S. filamentosa*; **E:** Similar in *S. macrolepis*. c, capsule of rodlet cell; cp, cytoplasmic extension of phagocyte; ec, epithelial cells; gg, goblet gland cells; m, mucus; mi, microvilli; n, nucleus of rodlet cell; np, nucleus of phagocyte; o, opening in the capsule; r, rods; rc, rodlet cells; arrow, apical thinner site of rodlet cell capsule.

to expose the cell-content to the alimentary tract lumen. As a result, RCs capsules often remain intact, embedded among the mucosa cells (Fig. 3B).

At this stage, entire RCs are often found in the gut, above the luminal epithelium, especially following pressure on the walls as a result of engulfing larger prey, and the contraction of the epithelial folds and secondary villi of the epithelium (Fig. 3C and inset). In several instances RCs were revealed in the stomach wall with damaged capsules and lobopodia-like protrusions of macrophages (phagocytes) penetrating into the cell (Fig. 3D, E).

Number of rodlet cells

The number of RCs observed per microscopic section differed in the various parts of the alimentary tract. For example, in cross-sections of the upper part of the stomach ca. 18-22 RCs/secondary villum were observed, whereas 35-50 RC/secondary villum were revealed in the central part of the stomach. On such sites 280-360 RCs were counted per 10 μ m cross-section of an 8 mm wide stomach in *Saurida tumbil* and *S. macrolepis*, as well as in *Synodus kaianus* while the lowest number, 80-140 per such cross-section of the stomach, were found in *Sy. variegatus* and *Trachinocephalus myops*. In all species studied, with increase in fish standard length (SL) an increase in the number of RCs per section of the alimentary tract was observed. For example, in juvenile specimens of *S. tumbil* and *S. macrolepis* of 50 mm SL, only 8-10 RCs/villum section were exposed in the stomach, and only a few dispersed cells were observed in the intestine. The lowest number of RCs was observed in the pyloric ceca and posterior tapering end of the stomach in all species studied.

Comparing the dimensions of sites occupied by the RCs in the tracts of adult fishes, with the surface dimensions of the epithelium in which they reside, revealed that in *Synodus variegatus* and *Trachinocephalus myops* ca. 5.0-7.0% of this epithelium is displaced by the RCs. In adult *Saurida tumbil* along 1 mm of mucosal epithelium of a secondary villum circa 20 ripe RCs were found, occupying 0.45-0.5 mm of the site, displacing almost 50% of the regular epithelial cells, and thereby blocking several pits of gastric tubular glands situated below this epithelium and opening into the lumen (Fishelson *et al.*, 2011).

DISCUSSION AND CONCLUSION

Rodlet cells have been found in all the 35 fish families studied for the presence of this remarkable cell (Manera and Dezfuli, 2004). The present study of lizardfishes (order Aulopiformes) adds the 36th family in which RCs have been found. Although studied for more than a century, the origin of these cells remains enigmatic. Today most investigators of RCs perceive them as "natural and endogenous" cells of tel-

eost fish, recently also expressed by Jordanova *et al.* (2007), describing them from the liver of *Salmo lentica*. However, despite this, the origin in the fish of these enigmatic cells remains unclear. As demonstrated by Fishelson and Becker (1999) for *Cyprinus carpio*, and recently by Reite and Evensen (2006) for some marine fishes, not in all specimens of a species the RCs are present.

The gross-morphological structure of RCs observed in the alimentary tract of several species of the studied lizardfishes of the genera *Saurida*, *Synodus* and *Trachinocephalus*, reveals them to be of two forms: one elongated-round with a tapering apical part adjacent to and facing the tract lumen. Such cells open directly into the lumen of the gut, liberating their rodlets and thereby closely resemble the RCs described in other bony fish by various authors, and summarized in the review by Manera and Dezfuli (2004).

However, the RCs in the gut of *Saurida filamentosa* and *S. argentea* from Australia differ strongly from this structure, not only by being bulbous; they are situated deep among the surrounding epithelial cells, closer to the basal membrane and not to the surface. As a consequence, the maturing RCs in this form develop a longer "neck" extending between the epithelial cells and their rodlets must move along a path between neighboring cells before arriving at the luminal surface of the intestine. This path is formed by separation of the surrounding membranes of the epithelium cells. Microvilli or other extensions of RCs into the gut lumen, as observed for cyprinid and other fishes (Iger and Abraham, 1977; Fishelson and Becker, 1999), were not revealed in the studied lizardfishes.

As shown by Leino (1974) and demonstrated in the present study, the fibrillar capsule of the RCs also includes numerous microtubules, 10-12 Å diameter, which extend parallel to the RCs long axis, interwoven with obliquely-running fibrillae. Such ca. 400-500 nm thick capsules have not been observed in any endogenous cell-types of fishes, especially not in the granulocytes, as otherwise argued by some researchers (e.g., Manera and Dezfuli, 2004). Such capsules possibly provide the RCs with improved elasticity when compressed between normal epithelial cells, while also protecting against the harmful actions of phagocytes and other immune-reactive elements. The micro-tubular structure of the capsule is of interest, in that EM cross-sections of such tubules at the RCs surface revealed opaque dots along the membrane, recognized in some instances as desmosomal junctions with neighboring epithelial cells (e.g., Imagawa *et al.*, 1998; Manera and Dezfuli, 2004). Kramer *et al.* (2005) also described desmosomes between RCs and surrounding cells; however, closer examination of their figures does not reveal such inter-cellular connections, typical for desmosomes (e.g., Delva *et al.*, 2009). In the present study, except for the opaque occurrence of such dots, groups of fibrils and cross-membrane connections typical for desmosomes were not observed, except for possible junctions of the cell-neck

to the neighboring epithelial cells. No desmosomal junctions of RCs with epithelial cells were observed in the head kidney of carp by Fishelson and Becker (1999).

With the formation of the intravascular opaque line, slightly larger tubules extend along the interior of the capsule, which seem to be part of the ER system of the cell, and possibly serve for metabolic activity, including cell growth. According to Bielek (2008), the origins of rodlet deposition lie in the vesicular extensions of the ER, which later form the rodlet sacs, with participation of Golgi elements. Is it possible that these sac membranes form a protective shield during development of the rodlets?

In several instances the rodlets were observed to possess two layers of different densities. According to Leino (1982), the inner core of such rodlets is rich in proteins, while the outer layer is rich in carbohydrates and proteins, possibly forming a protective (glycoprotein) layer of the rodlet.

Concomitant with growth in number and dimensions of rodlets in the RCs, the cell-nucleus decreases in dimensions and changes in form, as if the rodlets have been “sucked-out” from a part of the nucleus body. The observed “anchoring” of rodlets in the nucleus would seem to be connected with this change.

Various functions have been ascribed to the RCs by various authors, of which the “immunological approach” is especially prominent, summarized in the review by Manera and Dezfuli (2004). Iger and Abraham (1997) recognized a proteolytic activity of RCs and their antiparasitic activity was noted by several authors (Reite, 1997; Manera and Dezfuli, 2004; Dezfuli *et al.*, 2007a, 2007b). Furthermore, Koponen and Myers (2000), studying the RCs in the freshwater bream, perceived a high expression of these cells as indicators of peak parasite occurrence. Kramer and Potter (2002), revealing RCs in the head kidney of *Xiphophorus maculatus*, argued that cells are transported “on demand” from this site to various infected organs of the fish. The basis for such thinking lay in the observed larger numbers of RCs in fish infected by helminthes and other parasites, as well as in fish artificially or naturally exposed to harmful xenobiotics. Reite (2005) defined the RCs as a “defending force” developed by evolution, to combat harmful interventions in fish, and Dezfuli *et al.* (2000) saw in them “inflammatory cells”, and even suggested the use of the number of RCs present as a biomarker for environmental quality of the studied fish. However, we can also perceive the high number of RCs/high number of parasites in the fish from precisely the opposite direction: namely, fish populated by parasites or influenced by alien substances possess a weaker immune-system (“flies flock to sick horses”), and as such they are more vulnerable to increased numbers of RCs initiators. Kramer and Potter (2003), revealing the occurrence of RCs in embryos and neonates of the live-bearing *Xiphophorus maculatus*, showed this as evidence for the endogenous origin of these cells. However, in vivipa-

rous fish the developing embryo and neonates are physiologically exposed to the mother-fish (matrotrophy), and in such cases the exchange of material, and possibly RCs-initiators, is to be expected. Together with this we should bear in mind that the observed high numbers of RCs in the mucosal epithelium constitutes a burden on the digestive and transport function of this cell layer, as they displace and occupy sites otherwise belonging to cells actively involved in food absorption. Alvarez-Pellitero (2008) in his paper on turbot notes that the mucosal epithelium constitutes the primary barrier that protects the fish internally from pathogens. The openings into the lumen by the numerous RCs at least partly impact this effective barrier. The observed “devouring” of RCs by phagocytes, also described by Richards *et al.* (1994), is of special interest when discussing the nature of these cells. Recently, studying the occurrence of piscidine, the antibacterial peptide in teleosts Silphaduang *et al.* (2006) following a study of ca. 15 fish species, claimed the exposure of this peptide in the RCs of gill in one of four studied specimens of the cichlid *Oreochromis niloticus*. Closer examination of their LM figures, however, does not enable to identify the marked cells as RCs.

Among all these debates, the study by Viehberger and Bielek (1982) is important in demonstrating that the rodlets are laden with DNA, and that this DNA presumably differs in configuration from the DNA of RCs nuclei; as are the studies by Barber and Westermann (1986a, 1986b), which, following histochemistry and DNA-gold labeling, suggested a dual origin of RCs, recognizing in the rodlets an invasive pathogen “of unknown phylogeny”, a position also discussed by Fishelson and Becker (1999). At this stage we can quote Bielek (2008), that: “The questions of function and nature of RCs are still in debate”; and Alvarez-Pellitero (2008), that “the precise role of this enigmatic cell deserves farther investigations”.

Revealing two different forms of RCs in the lizardfishes, one elongated, as observed also in other fish families, and a new, bulbous, “peach-like form”, as observed in the Australian species of lizardfishes, *Saurida filamentosa* and *S. argentea*, raises a new question: are we dealing with a variation of one form, or are we in fact looking at two different types that await farther studies?

Acknowledgments. - The authors wish to thank Dr. Ofer Gon (South Africa) for gifts of fishes that served for the study. We also thank Dr. Izhak Brickner for the histological sections, Yakob Delarea for EM work, Varda Wexler for help in artwork, and Naomi Paz for editing the manuscript. The authors are grateful to the anonymous referees for their constructive remarks.

REFERENCES

- AGULLEIRO B., ZUASTI A. & LOZANO M.T., 1986. - Rodlet cells, gamonts and differentiating microgamonts of Apicomplexa in teleosts. *Acta Microsc.*, 9: 23-30.

- ALVAREZ-PELLITERO P., 2008. - Immunological responses of turbot (*Scotophthalmus maximus* (L.)) (Pisces, Teleostei). *Fish Shellf. Immunol.*, 25: 358-372.
- BARBER D.L. & MILLS WESTERMANN J.E., 1986a. - The rodlet cells of *Semotilus atromaculatus* and *Catostomus commersoni* (Teleosts): Studies on its identity using histochemistry and Dnase I-gold, Rnase A-gold and S1 nuclease gold labeling techniques. *Can. J. Zool.*, 64: 805-813.
- BARBER D.L. & MILLS WESTERMANN J.E., 1986b. - Comparison of DNA of the nuclei of rodlet cells and other cells in the chub *Semotilus atromaculatus*: hybridization *in situ*. *Can. J. Zool.*, 64: 801-804.
- BARBER D.L., MILLS WESTERMANN J.E. & JENSEN D.N., 1979. - New observations on the rodlet cell (*Rhabdospora thelohani*) in the white sucker, *Catostomus commersoni*: LM and EM studies. *J. Fish Biol.*, 14: 277-284.
- BIELEK E., 2002. - Rodlet cells in teleosts: new ultrastructural observations on the distribution of the cores in trout (*Oncorhynchus mykiss*, *Salmo trutta* L.). *J. Submicrosc. Cytol. Pathol.*, 34: 271-278.
- BIELEK E., 2008. - Membrane transformation in degenerating rodlet cells in fishes of two teleostean families (Salmonidae, Cyprinidae). *Anat. Rec.*, 291: 1693-1706.
- DELVA E., TUCKER D.K. & KOWALCHYK P., 2009. - The Desmosome. *Cold Spring Harbor Perspect. Biol.*, 1: a002543. 10 p.
- DENSMORE L.C., SMITH S.A. & ODENKIRK J., 2007. - Rodlet cells in tissues of the northern snakehead (*Channa argus*) from Potomac River. *IAAAM Conf. Proc.*: 1.
- DEZFULI B.S., SIMONI E., ROSSI R. & MANERA M., 2000. - Rodlet cells and other inflammatory cells of *Phoxinus phoxinus* infected with *Raphidascaris acus* (Nematoda). *Dis. Aquat. Org.*, 43: 61-69.
- DEZFULI B.S., CAPUANO S., SIMONI E., PREVIATI M. & GIRARI L., 2007a. - Rodlet cells and sensory systems in zebrafish (*Danio rerio*). *Anat. Rec.*, 290: 367-374.
- DEZFULI B.S., GIARI L. & SHINN A.P., 2007b. - The role of rodlet cells in the inflammatory response in *Phoxinus phoxinus* brain infected with *Diplostomum*. *Fish Shellf. Immunol.*, 23: 300-304.
- FISHELSON L. & BECKER K., 1999. - Rodlet cells in the head and trunk kidney of the domestic carp (*Cyprinus carpio*): enigmatic gland cells or coccidian parasites? *Naturwissenschaften*, 86: 400-403.
- FISHELSON L., GOLANI D., RUSSELL B. & GOREN M., 2011. - Comparative morphology and cytology of the alimentary tract in lizardfishes (Teleostei, Aulopiformes, Synodontidae). *Acta Zool.*, doi: 10.1111/j.1463-6395.2011.00504.x, 1-10.
- GOLANI D. & BEN-TUVIA A., 1995. - Lessepsian migration and the Mediterranean fishery of Israel. In: Conditions of the World's Aquatic Habitat (Armantrout N.B., ed.), pp. 1279-1289. Proc. World Fish. Congr. Theme 1. Oxford: Oxford & IBH Pub. Co.
- IGER Y. & ABRAHAM M., 1997. - Rodlet cells in the epidermis of fish exposed to stressors. *Tissue Cell*, 29: 431-438.
- IMAGAWA T., KITAGAWA H. & UEHARA M., 1998. - An association between the rodlet cells and the vascular endothelial cells in the head kidney of carp, *Cyprinus carpio* L.: ultrastructural observations. *J. Fish Dis.*, 21: 153-157.
- JORDANOVA M., MITEVA N. & ROCHA E., 2007. - A quantitative study of hepatic eosinophilic granule cells and rodlet cells during the breeding cycle of Ochrid trout, *Salmo lenticus* Kar. (Teleostei, Salmonidae). *Fish Shellf. Immunol.*, 23: 473-478.
- KOPONEN K. & MYERS M.S., 2000. - Seasonal changes in intra- and interorgan occurrence of rodlet cells in freshwater bream. *J. Fish Biol.*, 56: 250-263.
- KRAMER C.R. & POTTER H., 2002. - Ultrastructural observations on rodlet-cell development in the head kidney of the southern platyfish, *Xiphophorus maculatus* (Teleostei: Poeciliidae). *Can. J. Zool.*, 80: 1422-1438.
- KRAMER C.R. & POTTER H., 2003. - Rodlet cells in the posterior intestine of embryos and neonates of two poeciliid species. *J. Fish Biol.*, 62: 1211-1216.
- KRAMER C.R., KRAMER A.J. & KONOVALOV A., 2005. - Rodlet cell distribution in the gall bladder epithelium of *Fundulus heteroclitus*. *J. Fish Biol.*, 67: 555-560.
- LAGUESSE E., 1895. - Sur le pancréas du crénilabre et particulièrement sur le pancréas hépatique. *Rev. Biol. Nord.*, 7: 343-363.
- LEINO R.L., 1974. - Ultrastructure of immature, developing, and secretory rodlet cells in fish. *Cell Tissue Res.*, 155: 367-381.
- LEINO R.L., 1982. - Rodlet cells in the gills and intestine of *Catostomus commersoni* and *Perca flavescens*: a comparison of their light and electronmicroscopic cytochemistry with that of mucus and granular cells. *Can. J. Zool.*, 60: 2768-2782.
- MANERA M. & DEZFULI B.S., 2004. - Rodlet cells in teleosts: a new insight into their nature and function (review paper). *J. Fish Biol.*, 65: 597-619.
- MAYBERRY L.F., MARCHIONDO A.A., UBELAKER J.E. & KAZIC D., 1979. - *Rhabdospora thelohani* (Laguesse 1895) (Apicomplexa): a new host and geographic records with taxonomic consideration. *J. Protozool.*, 26(2): 168-178.
- REITE O.B., 1997. - Mast cells/eosinophilic granule cells of salmonids: staining properties and responses to noxious agents. *Fish Shellf. Immunol.*, 7: 567-584.
- REITE O.B., 2005. - The rodlet cells of teleostean fish: their potential role in host defense in relation to role of mast cells/eosinophilic granule cells. *Fish Shellf. Immunol.*, 19: 253-267.
- REITE O.B. & EVENSEN Ø., 2006. - Inflammatory cells of Teleostean fish: a review focusing on mast cells/eosinophilic granule cells and rodlet cells. *Fish Shellf. Immunol.*, 20: 192-208.
- RICHARDS D.T., HOOLE D., ARNEE C., LEWIS J.W. & EWENS E., 1994. - Phagocytosis of rodlet cells (*Rhabdospora thelohani* Laguesse, 1895) by carp (*Cyprinus carpio*) macrophages and neutrophils. *Helminthologia* (Bratislava), 31: 29-33.
- SCHMACHTENBERG O., 2007. - Epithelial sentinels or protozoan parasites? Studies on isolated rodlet cells on the 100th anniversary of an enigma. *Rev. Chiliana Hist. Nat.*, 80: 55-62.
- SILPHADUANG U., COLORNI A. & NOGA E.J., 2006. - Evidence for wide spread distribution of piscidin antimicrobial peptides in teleost fishes. *Dis. Aquat. Org.*, 72: 241-252.
- THÉLOHAN P., 1892. - Sur les sporozoaires indéterminés parasites des poissons. *J. Anat. (Paris)*, 28: 163-171.
- VIEHBERGER G. & BIELEK E., 1982. - Rodlet-cells: Gland cells or protozoan? *Experientia*, 38: 1216-1218.

Reçu le 10 mars 2011.

Accepté pour publication le 9 juin 2011.